cc sent.

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Dear Bruce--

Esther and I are taking a few days holiday in Mendocino County, and are happy to have some time, as this evening, to catch up on our more personal coreespondence -- as you must know already, we are shamelessly bad at this.

Thinking back over your visit, I have a sense of very deep regret over having seen so little of you, and especially of having failed to recapture that exultant spirit of planning and doing experiments with you that I enjoyed so muchbefore. True, this has been a difficult time, and I was only just beginning to catch up well enough to enjoy the lab more as you were leaving, but I can only remark how I could have been so unwise or so ineffective as to put first things second. But, believe it or not, this has changed very much for the better and I have been able to spend more hours in the lab than elsewhere, and to follow through on my own programs, as well as vet all the others going on. So I am just terribly sorry that we were out of phase. One important help on this has been the way that Elliott Levinthal has taken over the immediate management of the exobiology work, which is now going along beautifully on the engineering side; also, a Dr. Hochstein (who did his Ph.D. on the metabolism of nicotine by soil bacteria with Syd. Rittenberg) is coming in a couple of weeks to direct the other side of that work. So now, exobiology need no longer take the disprpportionnate part of my time that it did before. Also, Haruko Nagaishi is working out to be a splendid assistant, and we are now occupying the space you had in "Lab I".

The Kleins (which means mainly Eva, for the actual lab work) spent most of their time surveying additional markers for linkage to the his-aromatic complex; there are still some dubious possibilities that have to be rechecked but we have to conclude that we have no evidence yet of any additional linkage groups. I will be following this up as extensively as possible, and we are putting in a fair effort into collecting more mutants. We were also interested in the question of "digressive co-transfer", i.e., the frequency with which unlinked markers, e.g., his_1^+ ind^+ --x - - gives a mixed progeny of +- and -+ . We had some serious technical difficulties, as you may imagine: the most reliable experiments were done by plating the --x mix on nutrient agar, then replica-plating the lawn to the test media with careful registration, and looking for congruencies. These were then confirmed by picking the , e.g., -+ outgrowths and demonstrating the co-incidence of +- cells. At a (high)DNA level that gave about 5% of the transformants as ++ (prototrophic) "congressive" cotransfers, there was about an equal proportion, in addition, of the digressive mixtures. These results should relate very directly to some of your findings, on the single cells -- which I hope you will soon be able to have in typescript. Marion Schafer had done some earlier experiments along these lines, but with a different and quite unreliable technique, and with erratic results.

In the course of these experiments, we had indirect evidence, from the inefficiency of recovery of transformants on replica platings at certain inoculum levels that again had to be explained by a disadvantage

or delay of the transformants (or rather, I would believe, of competent cells). We therefore plated out some --x experiments and found, as we should have inferred long since, that the transformant clones can be detected rather efficiently as among the small colonies on nutrient agar. In combination with the selection by penicillin, this now gives us the most convenient method to study the events immediately after --x, and we are now deep in this. So far, every transformant clone has contained the 000 residue also, and we should believe that your isolated that did not must already have segregated.

There would also be some detail to report, that I cannot conveniently summarize as the picture is not yet very simple, on the kinetics of transformation, as functions of time, concentration and method of preparation of the DNA. There is some suggestion of a lag of about 90 seconds (interval of exposure to DNA before DNAse is added) before any transformants are produced, but we have not succeeded in filtration or temperature shift experiments in demonstrating any preliminary phase of attachment of DNA to cells still sensitive to DNAse. Mei has shown that no reaction occurs of DNA with cells at 0° C., and the optimum temperature is probably about 40-43°. The reaction is also inhibited by azide. Cell competence is destroyed, without much loss of total viability, by treatment with periodate, and it looks as if periodate has no effect immediately after DNA uptake, so I doubt if this is a differential susceptibility of competent cells to killing by periodate. We have also seen a surprising inactivation of DNA to rather higher concentrations of periodate, which is reminescent of McCarty's finding of the effect of ascorbogenic peroxide. It is not obvious what the chemical basis of these effects should be.

Perhaps the most exciting findings are just the persistence of some facts you already know -- Gan has repeated the CsCl fractionation of markers, which correlates quite nicely with the melting points, and Gan and Gene have quite good evidence of the breakage of the his-aromatics linkage complex by mechanical stirring of the DNA. It seems certain that the DNA as now prepared is quite heterogeneous, but we have not been able to conserve larger pieces (as reflected in linkage values) by more careful preparation -- as we have been doing in following up the experiments on the behavior of the DNA in transformant cells. I have to say that we have not been pushing that as hard as it should be, but I felt these other questions had to be cleared up first, and we are just now in a much better position to go back to it. Walter Bodmer is just getting underway, after his preliminary apprenticeship, on the enzymic inactivation of DNA, and is getting a good deal of help on this from Bill Razzell at the Syntex Institute (which is now flourishing, Boris included). Esther will give you her own account of her experiments, which involved so much bookkeeping that we could just now get a clear picture of their implications.